ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2016; 2(7): 80-85
www.allresearchjournal.com
Received: 11-05-2016
Accepted: 12-06-2016

Mistry Zoya
Department of Biotechnology, Veer Narmad South Gujarat University, Surat, India.

More Bhikhu
Department of Biotechnology, Veer Narmad South Gujarat University, Surat, India.

Shah Gaurav
Department of Biotechnology, Veer Narmad South Gujarat University, Surat, India.

Correspondence
Mistry Zoya
Department of Biotechnology, Veer Narmad South Gujarat University, Surat, India.

Anti-dandruff activity of synthetic and herbal shampoos on dandruff causing isolate: *Malassezia*

Mistry Zoya, More Bhikhu and Shah Gaurav

Abstract
Dandruff is the major problem for many people in today's world. Many anti-dandruff shampoos, whether synthetic or herbal, and lotions are marketed to combat the problem of dandruff. The present study deals with testing the effect of different synthetic and herbal shampoos on dandruff causing fungus isolate *Malassezia*. Isolation of *Malassezia* was carried out by using Dixon and Sabouraud Dextrose Agar medium. Total six synthetic and two herbal shampoos were selected to check the effectivity against *Malassezia* by agar cup method. Different concentrations of shampoos were taken to check the Minimum Inhibitory Concentration (MIC). All the selected shampoos were proved to be effective as they all showed the inhibition against *Malassezia*. Synthetic shampoos were proved to be more effective compared to herbal shampoos. The highest zone of inhibition was obtained by Cipla-8X while minimum inhibition was observed by Nature's Essence.

Keywords: Dandruff, *Malassezia*, Synthetic shampoos, Herbal shampoos

1. Introduction
Dandruff is a chronic scalp condition characterized by scaling, itching and redness of the scalp. It occurs when scalp sheds epidermal cells in large clumps. The skin of scalp renews itself about once a month. Usually, scalp sheds dead cells in nearly invisible way, but sometimes cell turnover becomes unusually rapid and dead cells are shed as visible flakes called dandruff (Loden & Wessman, 2000) [12]. Dandruff is a major cosmetic problem that poses very great public health concern both in developed and developing countries (Krishnamoorthy *et al*., 2006) [11].

According to the symptoms dandruff is classified into two types – Dry (common) and Oily. Dry dandruff also known as *Pityriasis simplex* is characterized by excessive formation of minute scales of white grayish or ashen color, accumulating on the scalp area. These scales are at first localized in the middle of scalp area and then spread towards parietal, frontal and occipital areas. In this type of dandruff, no excessive hair loss is observed. The other type of dandruff is called oily dandruff or *Pityriasis steatoides*. It arises on the scalp skin with varied intensity of sebum production. Inflammation of varied intensity develops on the scalp skin along with the appearance of oily scales of dirty yellow colour that can form lesions. Hairfall is common; it may also exacerbate androgenetic alopecia. The most common site affected by this type of dandruff is scalp, but it can occur between eyebrows, along the side of nose, behind the ears, over the breastbone and sometimes in the armpits (Nowicki, 2006) [14].
Dandruff scale is a cluster of corneocytes, which have retained a large degree of cohesion with one another and detach as such from the surface of the stratum corneum. In the physiological spectrum of scaling about 487,000 cells/cm^2 get released normally after detergent treatment and their number goes upto 800,000 cells/cm^2 during dandruff and seborrhoeic dermatitis.

Dandruff can almost be controlled and effectively treated, but the treatment of dandruff may take a little patience and persistence. In general, daily cleansing with a gentle shampoo to reduce oiliness and skin cell buildup can often help mild dandruff. When regular shampoos are not effective, dandruff shampoos can be used. Also, dandruff shampoos are not all alike, and one may need to experiment until they find the one which best suits them. The formulations must be suitable for hairy regions and combat the dandruff conditions. It is therefore essential that these formulations have accepted pharmaceutical properties at the cosmetological level. Different types of formulations like shampoos, creams, lotions, emulsions, hair oils and other cosmetic formulations are readily available in the market that

are used to control dandruff. These formulations include therapeutic use of anti-dandruff agents that are classified into three groups according to their mechanism of action;

1. Fungicidal substances: e.g., zinc pyrithione and imidazoles.
2. Cytostatic substances: e.g., tar, selenium sulfide and octopirox.
3. Keratolytic substances: e.g., salicyclic acid and sulfur compounds (Adamski, 1995) [1].

Malassezia (formerly known as *Pityrosporum*) is a monophyletic and unipolar lipophilic yeast. It is naturally found on the skin surfaces of many animals, including humans and associated with a variety of conditions including dandruff, atopic eczema/dermatitis, pityriasis versicolor, seborrheic dermatitis and folliculitis (Ashbee and Scheynius, 2010) [2]. It is a part of natural body flora. It lives quietly and unnoticed on our body, usually without our being aware of its presence unless stress, illness, antibiotics or other unfavorable conditions upset the natural balance of our body's immune response. The genus *Malassezia* consists of seven species (Gueho *et al*., 1996) [8]. *M. furfur* is restricted to the human host, *M. sympodialis* is a cat and human pathogen (Bond *et al*., 1996) [3] and *M. pachydermatis*, the causative agent of canine otitis externa (Klein *et al*., 1996) [10] is also a human pathogen (Bond *et al*., 1996) [3]. The remaining four species viz., *M. globosa, M. obtusa, M. restricta* and *M. slooffiae* were recently isolated from infections that were previously believed to be caused by *M. furfur, M. sympodialis* or *M. pachydermatis* in origin (Gueho *et al*., 1996) [8].

A shampoo may be described as a cosmetic preparation required for the washing of hair and scalp, packed in a form which is convenient for use. The word shampoo comes from the french word meaning Beard soap (Nanda *et al.*, 2006) [13]. Its primary function is to cleanse the hair of accumulated sebum, scalp debris and residues of hair-grooming preparations. The added functions of shampoo include lubrication, conditioning, body building, prevention of static charge build up, medication and so on. Two types of anti-dandruff shampoos are available commercially;

1. Synthetic anti-dandruff shampoos (based on ingredients of chemical origin)
2. Herbal anti-dandruff shampoos (based on plant ingredients)

2. Methods & Materials

2.1 Isolation of *Malassezia* species

2.1.1 Sample Collection: Flakes or scales were collected from scalp by partitioning the hair with a sterile comb and scrapping approximately one inch area using a sterile blunt scalpel. The specimen was then transferred into a dark sampling paper to prevent exposure to sunlight. The samples were inoculated over the surface of Sabouraud Dextrose Agar (SDA) plus olive oil and Dixon's Agar (DA) which was incorporated with chloramphenicol to avoid bacterial contaminants into sterile petri plates. The plates were then incubated at 30 °C for 7 days, which were observed regularly.

2.2 Sample Analysis

2.2.1 Direct microscopy: A drop of 10% KOH was added onto a clean slide containing the smear of sample and covered using a coverslip. The sample was then heated over a Bunsen burner to remove bubbles. The slides were viewed under 40X objective lens (Cheesbrough, 2000; Crespo *et al*., 2000; Kindo *et al*., 2004) [5, 6, 9].

2.2.2 Culture: The collected samples were cultured on SDA or DA media which was incorporated with chloramphenicol to get rid of the bacterial contaminants. Small amounts of the samples collected were introduced into petri dishes containing the media using sterile forceps. The petri dishes were labeled accordingly and incubated at 30 °C for 7 days.

2.2.3 Biochemical Tests

Catalase Test: Catalase test was carried out to ascertain the presence of *Malassezia* species as it is catalase positive, except *M. restricta* which is catalase negative. 3 mL of 3% hydrogen peroxide (H_2O_2) solution was poured into a test tube. Several colonies of the isolated fungal colonies were immersed into the test tube using a sterile glass rod.

2.2.4 Esculin Hydrolysis Test: The medium used was bile esculin agar slant which is a nutrient agar-based medium containing 0.1% esculin and 10% bile salts, and allowed to solidify as a slant. The bile salt inhibits some bacteria, and also shows the ability to grow in the presence of bile salts represents a second test use for the medium. An inoculum from a pure culture was aseptically transferred into a sterile tube of bile esculin agar and streaked along the slant. The inoculated tube was incubated at 30 °C for 24 h and the result was determined.

2.2.5 Gram's staining: A smear of pure culture was prepared and Gram staining was carried out to study the morphology of the yeast cells.

2.3 Anti-fungal Activity

2.3.1 Agar Cup Method

Agar Cup method was performed to check the antifungal activities of shampoos (Table 1). Dixon's media was used to prepare plates. Two days prior inoculated culture of *Malassezia* species in Dixon's broth was maintained to be used for this assay. 500 µL of culture suspension was spread on the petri plates. Each plate contained a well of 0.6 cm in diameter in which 100 µL of 100% concentrations of different shampoos, natural extracts, oils and lotion were added using a micro-pipette. Experiments were done in duplicates with suitable controls.

Table 1: List of Anti-dandruff shampoos used during the research work

Sr. No.	Name of Anti-dandruff shampoo	Active Ingredients	Manufacturer
1	Head & Shoulders	Zinc Pyrithione	Procter & Gamble
2	All Clear	Zinc Pyrithione	Hindustan Unilever
3	Garnier Fructis Fortifying	Zinc Pyrithione	L'Oreal India
4	Pantene Pro-V	Zinc Pyrithione	Procter & Gamble
5	Salisia-KT	Salicylic Acid Ketoconazole	Ajanta Private Limited
6	Cipla-8X	Zinc Pyrithione Ciclopirox	Cipla Limited
7	Himalaya Herbals Anti-dandruff Shampoo	Tree Tea Oil	Himalaya Herbals Health care
8	Nature's Essence Kesh Vishesh	Neem mla	Magikle Pharma Private Limited

2.3.2 Minimum Inhibitory Concentration (MIC)
MIC was performed in Dixon's agar plates by agar cup method. 24 h active culture of the test organisms were used for this study. The culture of *Malassezia* in Dixon's broth was used for inoculation and incubated at 30°C for 24 h. The same protocol was followed as mentioned above. The concentrations to check the MIC for a given sample used were 2.5%, 5%, 10%, 15%, 20%, 25%, 50%, 75% and 100% (v/v). The dilutions were done using sterile distilled water. Experiments were performed in duplicates with suitable controls.

2.3.3 Zone of Inhibition (ZOI)
ZOI was done on Dixon's plates by agar cup method. 24 h active culture was spread using a sterile glass spreader over the surface of Dixon agar. All the shampoos were dissolved in sterile distilled water. The same procedure was used that is mentioned above to check the zone of inhibition and the plates were incubated at 30 °C for 18 h. After incubation, the plates were observed. The inhibition zone was measured using a zone measuring scale and the results were recorded.

3. Results & Discussion
3.1 Isolation of *Malassezia*
The collected samples were streaked over the surface of SDA and 5 different colonies were obtained. Furthermore, these colonies were individually streaked in separate SDA plates. According to the morphological characteristics, *Malassezia* was identified and further inoculated in Dixon's agar (Plate 1 (a)). The sample was analyzed on the basis of direct microscopy of the collected sample of hair and scalp containing dandruff which showed hyphae and conidiospores exhibiting the characteristic "spaghetti and meatball" appearance in KOH preparation (Plate 2 (c)). Gram's staining showed ovoid shaped yeast cells (Plate 1 (b).
Furthermore, catalase test was performed which showed active bubbling when several isolated colonies of fungi was inoculated in a test tube containing 3 mL of 3% H_2O_2, therefore indicating a positive result (Plate 1 (d)). Also, Esculin hydrolysis test was performed in which pure culture was streaked on the slant of Bile esculin agar and abundant growth indicated positive test for growth in the presence of bile. Esculin hydrolysis was observed when the medium changed to chocolate brown color (Plate 1 (e)).

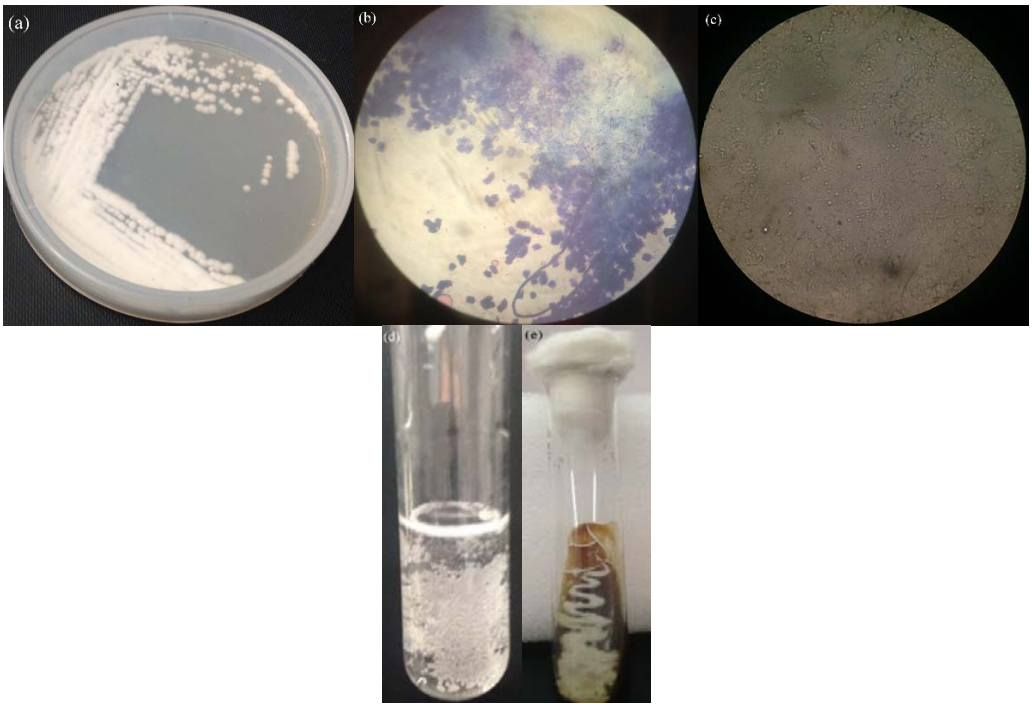


Plate 1: Isolation of *Malassezia*; (a) Pure culture of *Malassezia*; (b) Gram stained ovoid shaped cells; (c) Direct microscopy using KOH showing sphagetti and meatball shape; (d) Catalase test showing active bubbling; (e) Bile esculin agar slant showing abundant growth.

3.2 Anti-fungal Activity
3.2.1 Agar Cup Method: Agar cup method was performed throughout the experiment for different samples of shampoos to check the inhibition levels (Table 2) of *Malassezia* species (Plate 2).

Table 2: List of Shampoos used and their respective zone size in diameter (in cm)

Name of the Shampoo	Zone of Inhibition in diameter (in cm)
Head & Shoulders	1.8
All Clear	2.3
Garnier Fructis Fortifying	1.8
Pantene Pro-V	1.9
Salisia-KT	1.0
Cipla-8X	2.6
Himalaya Herbals	2.5
Nature's Essence	0.4

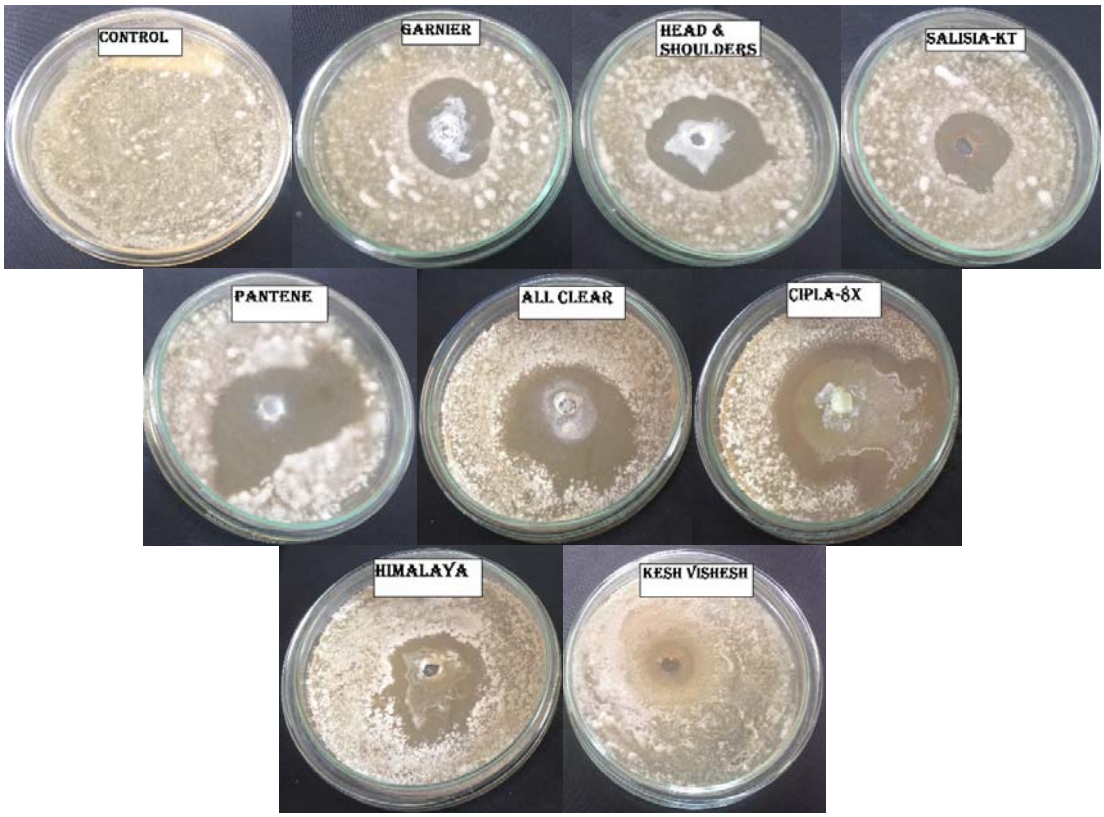
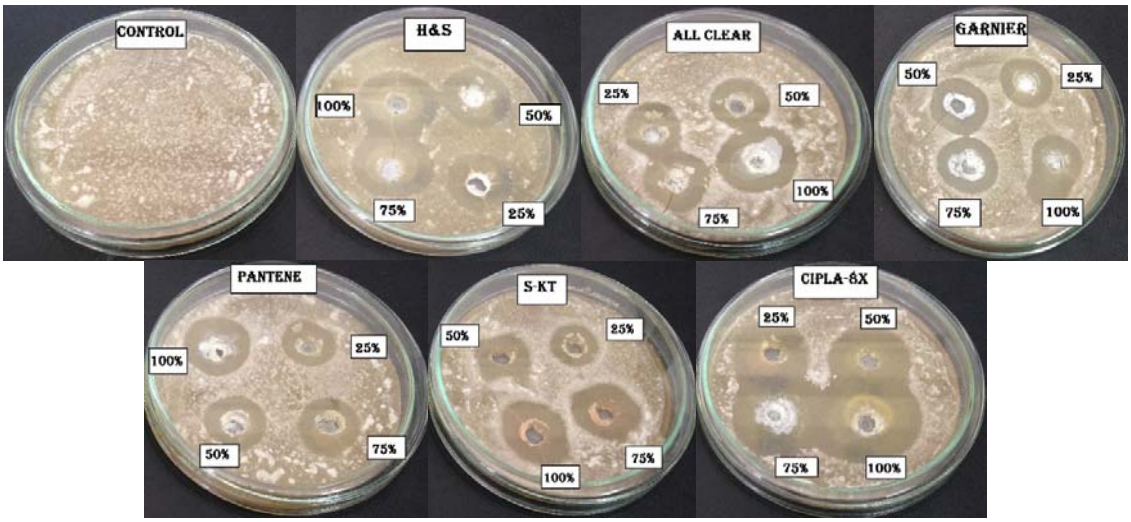


Plate 2: Antifungal activity of different shampoos on *Malassezia*

3.2.2 Minimum Inhibitory Concentration (MIC): The MIC was performed to check at which percentage of different concentrations of various shampoos (Table 3) were able to inhibit the growth of *Malassezia*. The results have been tabulated below:

Table 3: Effect of different concentrations of different samples of shampoos on *Malassezia* and the measurement of their zone size in diameter (in cm)

	Zone of Inhibition in diameter (in cm)								
Name of the Shampoo	2.5%	5%	10%	15%	20%	25%	50%	75%	100%
Head & Shoulders	-	-	0.2	0.3	0.4	0.6	0.7	0.8	1.0
All Clear	-	0.1	0.4	0.4	0.5	0.5	0.7	0.8	1.0
Garnier Fructis	-	0.1	0.4	0.5	0.6	0.7	0.9	1.1	1.5
Pantene Pro-V	-	0.1	0.2	0.4	0.6	0.6	0.8	0.9	1.1
Salisia-KT	-	0.1	0.2	0.4	0.5	0.6	0.8	0.9	1.1
Cipla-8X	-	-	0.2	0.3	0.4	0.8	1.0	1.2	1.5
Himalaya Herbals	-	-	-	-	-	-	0.6	0.8	1.0
Nature's Essence	-	-	-	-	-	-	0.1	0.2	0.5



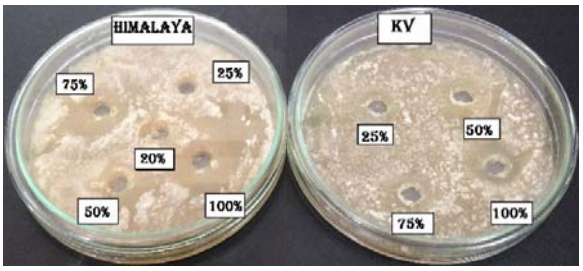


Plate 3: Effect of different concentrations of different shampoos on *Malassezia*

- Different concentrations (2.5%, 5%, 10%, 15%, 20%, 25%, 50%, 75% and 100%) of shampoos (Head & Shoulders, All Clear, Garnier, Pantene, Salisia-KT, Cipla-8X, Himalaya and Nature's Essence) were prepared to check the efficiency of shampoo in terms of inhibition (Plate 3).
- The maximum inhibition was observed at 100% for all the shampoos used during the experiment. For Head & Shoulders, the zone size was found to be 1.0 cm, for All Clear, it was 1.0 cm, for Garnier 1.5 cm, for Pantene 1.1 cm, for Salisia-KT 1.1 cm , Cipla-8X 1.5 cm, Himalaya 1.0 cm and Nature's Essence, the zone size was found to be 0.5 cm (Plate 3). showed that zinc pyrithione in Head & Shoulders, All Clear, Garnier, Pantene and Cipla-8X. Cipla-8X contains ciclopirox as well, as one one the ingredients required in the treatment of dandruff.
- Salisia-KT contains ketoconazole and salicylic acid which has been shown to improve the visible symptoms of flaking and restore the underlying skin condition.
- Tea tree oil is the main constituent in Himalaya shampoo for treating dandruff. Sharma, S. *et al*., (2013) [16] showed that Tea tree oil which is a mixture of hydrocarbons and terpenes, consisting of almost 100 substances gave good antimicrobial property and is also attributed primarily to the major component, terpinen-4-ol. Tea tree oil represents a sound alternative for patients with dandruff who prefer a natural product and who are willing to shampoo their hair daily.

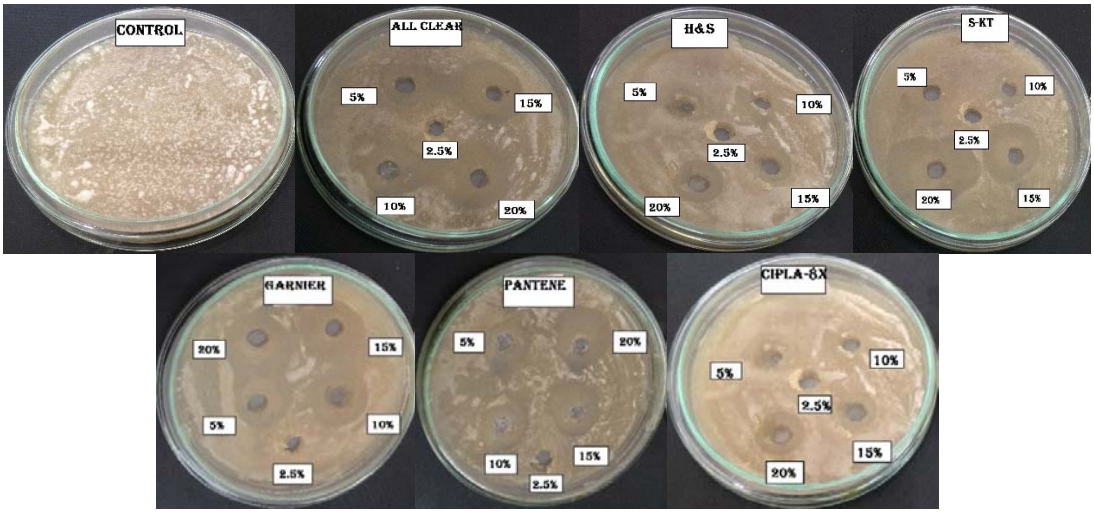


Plate 4: Effect of different concentrations of different shampoos on *Malassezia*

- The minimum inhibition was different for all the above used samples (Plate 4). For shampoos, like Head & Shoulders, the minimum inhibition was found to be at 10% (0.2 cm), for All Clear, it was at 5% (0.1 cm), for Garnier at 5% (0.1 cm), for Pantene at 5% (0.1 cm), for Salisia-KT at 5% (0.1 cm), for Cipla-8X at 10% (0.2 cm), for Himalaya at 50% (0.6 cm) and for Nature's Essence, it was observed to be at 50% (0.1 cm).

4. Conclusion

Anti-dandruff shampoos are widely accepted today to get rid of dandruff. These anti-dandruff formulations include therapeutic use of anti-dandruff agents that are classified into three groups according to their mechanism of action. These include Fungicidal substances (Zinc pyrithione, Ketoconazole etc.), Cytostatic substances (Selenium sulfide, tar etc.) and Keratolytic substances (Salicylic acid, sulfur derivatives etc.). Anti-dandruff products containing these agents work symptomatically and often recurrence of dandruff is observed after the treatment has been stopped, which is the mostly frustrating. Antifungal activity against the dandruff causing isolates with MIC values ranging from 2.5%, 5%, 10%, 15%, 20%, 25%, 50%, 75% and 100%. The maximum inhibition was eventually observed at 100% for all shampoo samples. The highest zone of inhibition was obtained by Cipla-8X. Moreover, Salisia-KT contained Salicylic acid and Ketoconazole which also gave satisfactory results. Herbal anti-dandruff shampoos were also found to be effective but their anti-dandruff effect was less compared to synthetic ones.

5. References

1. Adamski Z. Role of Malassezia furfur (Pityrosporum ovale, Pityrosporum orbiculare) in dermatology. Postepy Dermatologii 1995; 12:351-454.
2. Ashbee HR, Scheynius A. *Malassezia*. In: Ashbee HR, Bignell EM, editors. Pathogenic yeasts. The yeast handbook. Berlin Heidelberg: *Springer-Verlag*, 2010, 209-230.
3. Bond R, Anthony R, Dodd M, Lloyd D. Isolation of *Malassezia sympodialis* from feline skin, Journal of Medical & Veterinary Mycology. 1996; 34:145.
4. Bulmer AS, Bulmer GS. The antifungal action of dandruff shampoos Mycopathologia 2000; 147:63-65.
5. Cheesbrough M. Fungal Pathogens. District Laboratory Practice in Tropical Countries Cambridge University Press Part 2000; 2:35-48.
6. Crespo VE, Ojeda AM, Vera AC, Crespo AE, Sanchez FF. *Malassezia globosa* as the causative agent of Pityriasis versicolor, British Journal of Dermatology. 2000; 143:799-803.
7. Currens J, Hutcheson PS, Slavin RG, Citardi MJ. Introduction to Scalp, Indian Journal of Dermatology. 2002, 165-168.
8. Gueho E, Midgley G, Guillot J. The genus *Malassezia* with descriptions of four new species. Antonie van Leeuwenhoek. 1996; 69:337-355.
9. Kindo AJ, Sophia SKC, Kalyani J, Anandan S. Identification of Malassezia species, Indian Journal of Medical Microbiology. 2004; 22(3):179-181.
10. Klein N, Gill M, Cunha B. Unusual organisms causing intravenous line infections in compromised host: II. Fungal infections, Infectious Diseases in Clinical Practice. 1996; 5:303-10.
11. Krishnamoorthy JR, Ranganathan S, Shankar G, Ranjith MS. Dano A Herbal Solution for Dandruff, African Journal of Biotechnology. 2006; 5(10):960-962.
12. Loden M, Wessman C. The anti-dandruff efficacy of a shampoo containing piroctone olamine and salicyclic acid in comparison to that of a zinc pyrithione shampoo, International Journal of cosmetic Science. 2000; 22:285-289.
13. Nanda S, Nanda A, Khar RK. Shampoos. In: Cosmetic Technology. 1st ed. Birla Publications Pvt. Ltd., Delhi, 2006, 354-378.
14. Nowicki R. Modern Management of dandruff Pol Merkur Lekarski 2006; 20(115):121-124.
15. Ranganathan S, Mukhopadhyay T. Dandruff: The most commercially exploited skin disease, Indian Journal of Dermatology. 2010; 255(2):130-134.
16. Sharma S, Upadhyay UM, Upadhyay SU, Patel T, Trivedi P. Herbal Armamentarium for the culprit dandruff, International Journal of Phytopharmacy Research. 2013; 4(1):23-28.